A NOTE ON THE PATHOLOGY OF LESIONS OF THE CORNEA AND SKIN IN ANIMALS EXPERIMENTALLY INFECTED WITH *T. RHODESIENSE*

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(Received for publication 2 November, 1910)

As mentioned in a previous paper* three goats and a horse infected with *T. rhodesiense* all developed an interstitial keratitis of a remarkably transient character.

GOAT 1. On the sixth day after inoculation the temperature rose to 107° F. and parasites appeared in the peripheral blood, one being found to a field (Zeiss objective DD, eye-piece 4). During the next five days parasites were present in the blood in small numbers, one to from twenty to fifty fields. Subsequently the temperature fell to 103° F., and except on the thirty-first day, when one trypanosome was seen in fifty fields, parasites were not again found in the blood until the day of death, which occurred on the fifty-fifth day after inoculation, when one trypanosome was present in fifty fields.

On the fifteenth day a distinct swelling of the skin and subcutaneous tissue was visible below the eyes and over the nasal bones. This condition became gradually more marked and persisted until the animal's death.

On the seventeenth day it was noticed that the lower portion of the left cornea was slightly milky in appearance. This cloudiness rapidly became more dense, until on the nineteenth day the whole of the lower two-thirds of the left cornea was densely opaque.

* Yorke. 'On the Pathogenicity of a Trypanosome (*T. rhodesiense*) from a case of Sleeping Sickness contracted in Rhodesia,' Ann. Trop. Med. and Parasitol., Vol. IV, p. 351, 1910.

Slight opacity of the right cornea was now apparent. On the twenty-fourth day both corneae were opaque. During the next week the opacity gradually disappeared, until on the thirty-second day the eyes appeared perfectly normal. On the thirty-seventh day the left cornea again became cloudy, and on the thirty-ninth was opaque. The following day slight opacity was observable in the other cornea. The left cornea remained densely opaque and the right slightly cloudy until the death of the animal.

GOAT 2. Parasites were first observed in the blood (one to fifty fields) on the fourth day after inoculation. In the course of the next thirty-three days they were found on ten occasions in very small numbers. During the last nine days no parasites were found. The animal died on the forty-sixth day. The temperature never rose above 104° F. Oedematous swelling of the integuments below the eyes and over the nasal bones appeared on the sixteenth day and persisted until death.

On the thirty-sixth day slight opacity of the left cornea was observed for the first time. The opacity rapidly spread from below upwards, until on the thirty-eighth day the left cornea was densely opaque in its lower two-thirds. On the thirty-ninth day the left cornea was found to be clearing, whilst there was a slight cloudiness of the cornea of the opposite eye. The following day the right cornea was opaque. During the next few days the opacity rapidly disappeared from both eyes, until on the forty-second day the left cornea was clear and the right almost clear. During the last four days the right cornea became slightly more cloudy; the left remained clear until death.

GOAT 3. Parasites (one to fifty fields) first seen in the blood on the eleventh day after inoculation. They were subsequently found in very small numbers on the twenty-first and thirty-ninth days. The animal died on the fifty-fourth day. Temperature remained between 103° and 104° F. Oedematous condition of the skin of the face apparent on the thirtieth day and persisted until death.

Slight cloudiness of the lower portion of the right cornea appeared for the first time on the thirty-ninth day. The opacity rapidly increased in extent and density, until on the forty-second day the whole cornea was densely opaque. The condition persisted until death. The left cornea was unaffected.

In none of the goats was any other pathological condition of the eye observed. The pupils dilated normally with atropine. There was no conjunctivitis nor injection of the sclerotic.

HORSE 1. Parasites were first found in the blood on the seventh day. Subsequently they were present regularly in small numbers until the animal's death on the thirty-eighth day.

Slight haziness of the left cornea was first apparent on the thirty-fifth day. The condition gradually increased in severity, until at the time of death the whole cornea was cloudy.

The right cornea was unaffected. The sclerotic of the left eye was slightly injected.

For the purpose of studying the histological appearances the eyes were removed at the time of death and small portions of the cornea fixed in various fluids, viz., formalin 10 per cent., Flemming's solution and mercuric chloride alcohol. The tissues were embedded in paraffin and the sections stained with Heidenhain's iron alum haematoxylin, Breinl's safranine methylene-blue orange tannin, Giemsa and van Gieson. The best results, so far as the staining of the parasites was concerned, were obtained by staining the tissue which had been fixed in Flemming's solution by either the iron alum haematoxylin or the safranine methylene-blue orange tannin method. In well-fixed sections stained by these methods the trypanosomes stained exceedingly well, and the different portions of the parasites were easily distinguished. On the other hand, in those which had been fixed with formalin, the parasites did not stain, or at the best were very indistinct.

For purposes of description the eight corneae examined may be grouped into four classes.

1. Those which were densely opaque at the time of death, viz., left cornea of Goat 1 and the right of Goat 3. In these the morbid changes were very marked. The pathological conditions in the two were somewhat different. In the left cornea of Goat 1 the lesions were entirely confined to the substantia propria, the anterior and posterior epithelial layers being unaffected. Scattered throughout the substantia propria, which was almost twice as thick as that of a normal eye, were small oedematous patches. These were situated for the most part in the anterior third of the substantia propria. In the distended interlamellar spaces were a few leucocytes (both

polymorphonuclear and mononuclear) and also large numbers of trypanosomes. In the deeper layers of the substantia propria there was marked cellular infiltration and also considerable vascular formation. These areas also extended in places into the anterior third reaching occasionally almost to the anterior homogeneous membrane. Trypanosomes were found scattered throughout this portion of the cornea and in certain spots they existed in enormous numbers.

Appearances somewhat similar to the above have been described by Morax* as occurring in the corneae of dogs and goats infected with *T. equiperdum*.

The state of affairs obtaining in the right cornea of Goat 3 represented a rather more advanced stage. The substantia propria was about three times as thick as that of a normal cornea, and exhibited similar changes to those described in the previous case, viz., markedly oedematous areas lying side by side with patches of intense leucocytic infiltration. There was also some extravasation of red cells from the newly-formed vessels. The morbid processes were not, however, confined to the substantia propria, but had involved the anterior epithelial layer, which in places was separated from the substantia propria by inflammatory exudation. In these regions the epithelial cells were degenerated and stained badly, and at some points were completely disintegrated.

In contradistinction to the previous case only few trypanosomes were found scattered throughout the substantia propria.

II. Those which were slightly opaque at the time of death, viz., right cornea of Goat 1 and of Goat 2 and the left of the horse. Here the lesions were limited to the substantia propria which was slightly increased in thickness. In the deeper layers near the corneo-sclerotic junction there was considerable cellular infiltration and slight vascular formation. Elsewhere the only change visible was a slightly oedematous condition with the occurrence of a few leucocytes and trypanosomes scattered throughout the interlamellar spaces.

III. The left cornea of Goat 2, which after being densely opaque had subsequently become perfectly clear again. The

* 'Manifestations oculaires au cours des Trypanosomiases,' Annales de l'Institut Pasteur, 1907, p. 47.

anterior and posterior epithelial layers were normal. The substantia propria was slightly thicker than normal and oedematous in places. Near the corneo-sclerotic junction there was considerable infiltration of polymorpho- and mono-nuclear leucocytes and some new vascular formation, but elsewhere only a few leucocytes were found. No parasites were seen.

IV. The left cornea of Goat 3 and the right of the horse, which had remained unaffected throughout the disease. In these nothing abnormal was observed microscopically.

A comparable condition of affairs was found to exist in the lesions of the skin of an infected rabbit.

Small pieces of skin were removed from the following different areas:—(*a*) Normal skin of back; (*b*) area recently affected in which the hair was being shed and the skin oedematous; (*c*) bald markedly oedematous area; (*d*) region which had been bald for some weeks and showed some signs of recovery. The thickening of the skin had largely disappeared and there was a slight re-growth of hair.

The methods of fixing and staining were those employed for the cornea.

The histological appearances presented by sections of skin from these different areas may be classified as follows:—

(*a*) No pathological changes and no parasites were found.

(*b*) Large numbers of trypanosomes were found lying in the oedematous interstitial spaces. There was also considerable cellular infiltration.

(*c*) In this region the skin was considerably thickened and markedly oedematous. Enormous numbers of trypanosomes were present. There was great cellular infiltration involving the hair follicles and glands. Many of the hair follicles had completely disappeared.

(*d*) Here no parasites were found. The skin was not distended with fluid and the cellular infiltration was only slight.

Judging from the appearance presented by this series of sections of the cornea and skin the course of events would seem to be somewhat as follows:—

In early lesions trypanosomes are present in the tissues, and as a result there is an oedematous condition of the part, and a more or less marked degree of leucocytic infiltration.

Later as the number of parasites increases the morbid condition becomes more accentuated. Large numbers of leucocytes are poured into the tissue and new vessels develop. After a time the parasites disappear and with their disappearance there is a tendency on the part of the tissue to recovery.

The rapidity with which these processes occurred in the cornea is very remarkable. One can hardly suppose that the changes in the right cornea of Goat 1 and the left of Goat 2, which from being densely opaque were after the interval of a few days apparently normal, had progressed so far as the formation of new vessels; yet, on account of the extreme opacity, there must have been very considerable cellular infiltration, although this might in part be explained by the presence of enormous numbers of parasites such as were seen in the left cornea of Goat 1, which was densely opaque at the time of death.

The fact that trypanosomes can multiply so readily in the tissue spaces and at the same time be either entirely absent from the blood, or present in very small numbers only, is one of considerable interest, although the explanation is not very obvious. Perhaps the tissue juices form a more favourable nidus for the growth of the parasites, or possibly in these situations they escape to some extent the action of certain anti-bodies which have been shown to exist in the blood. Whatever the cause may be, the observation illustrates in what manner it is possible for an animal to be heavily infected and at the same time present no parasites in the peripheral circulation.

The drawings were done by Miss A. M. Brookfield.

DESCRIPTION OF PLATES.

PLATE XXV.

Left cornea of Goat 1.—Fixed with Flemming's solution. Stained by Heidenhain's iron alum haematoxylin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 8 compensating ocular (Zeiss). Magnification 1,000 diameters.

Considerable oedematous infiltration of the substantia propria. In the distended interstitial spaces are large numbers of trypanosomes. The parasites are cut in various directions, some transversely and others obliquely, whilst others are lying horizontally in the plane of the section.

There is also considerable infiltration of polymorphonuclear and mononuclear leucocytes. Two small blood vessels are seen in the lower portion of the drawing.

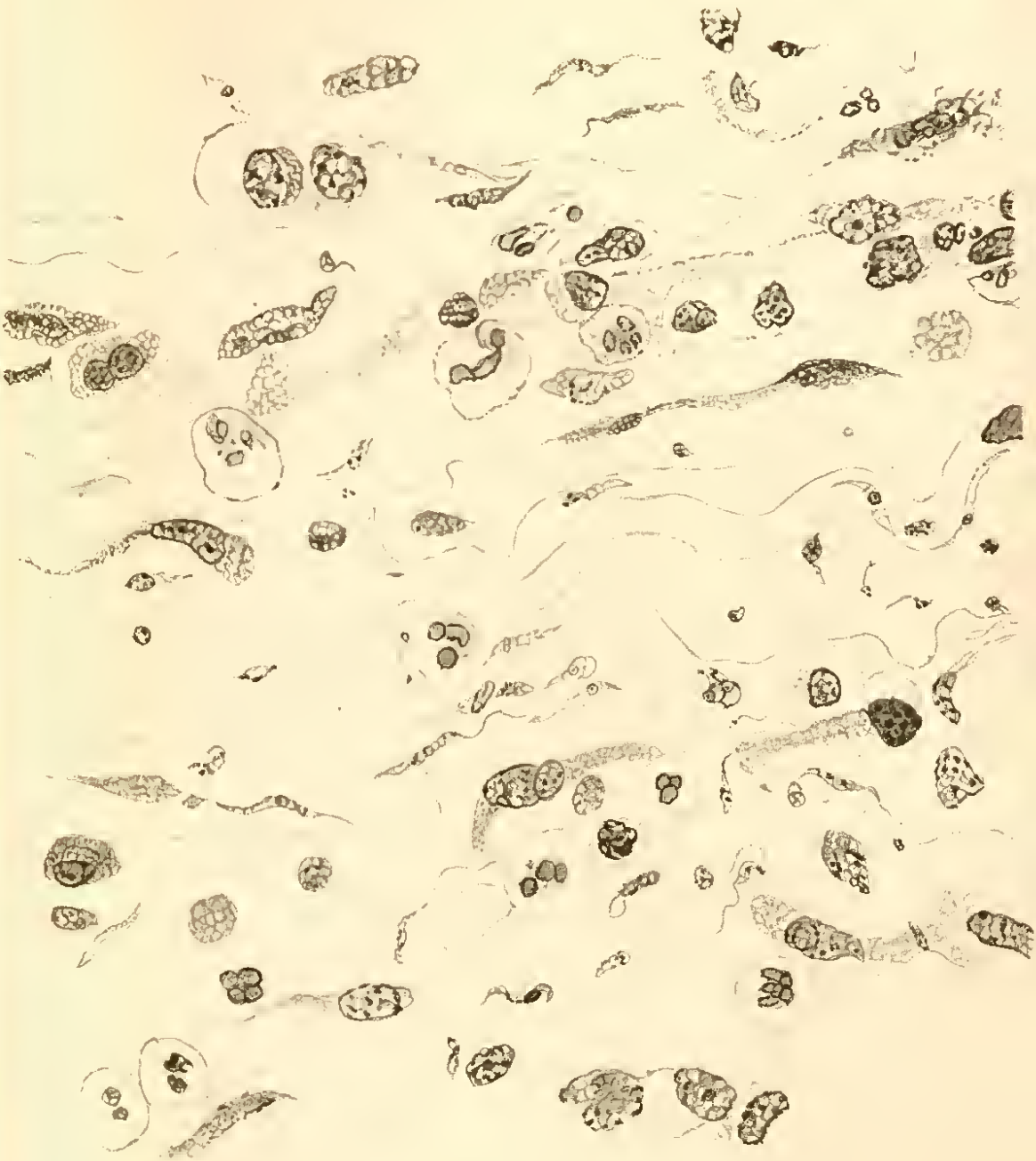

Plate XXVI.

Right cornea of Goat 3.—Fixed with Flemming's solution. Stained by Breinl's safranine methylene-blue orange tannin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 12 compensating ocular (Zeiss). Magnification 1,500 diameters.

The drawing represents a small portion of an area of marked cellular infiltration and vascularisation. A small vessel runs vertically across the field. Trypanosomes, polymorpho- and mono-nuclear leucocytes are seen lying between the fibres of the substantia propria.